Degenerative Human Intervertebral Disc Cells Exhibit an Inflammatory Phenotype upon Interleukin-17 Stimulation

INTRODUCTION.

Interleukin-17 (IL-17) is a proinflammatory cytokine expressed by CD4+ Th17 cells that may be involved in autoimmune disease. Pathological intervertebral disc tissue (IVD) exhibits inflammatory histopathology involving the presence of infiltrating macrophages and lymphocytes with various molecular mediators of inflammation produced by these cells (TNFα, IL-1α, and IL-1β) and IFNγ. Cultured IVD cells respond to exogenous TNFα or IL-1β with elevated PGE2, IL-6, and proteolytic enzyme release, suggesting a biological role for the presence of these cytokines in degenerative tissues. IL-17 may be variably regulated by the simultaneous presence of the Th1 lymphocyte product IFNγ. Indeed, IL-17 synergizes with IFNγ in eliciting inflammation. Nevertheless, the biological response of IVD cells to IL-17 and IFNγ has not been studied, and may be important given the cellular immunophenotype in the degenerative IVD. The objective of this study was to test for isolated and synergistic effects of IL-17 and IFNγ on production of inflammatory mediators by cells derived from degenerative human IVD tissue. Evaluating a biological response of IVD cells to these cytokines is critical in understanding the inflammatory processes in IVD pathology.

MATERIALS AND METHODS.

Cell Isolation. Surgical IVD tissues were procured from patients undergoing surgery for degenerative disc disease (n = 9 patients and 25 levels, age 60 ± 7 years). The anulus fibrosus (AF) and nucleus pulposus (NP) regions were mechanically separated and cells were isolated by enzymatic digestion. Cells were plated at 50,000 cells per well in 48-well plate and overlaid with 250 µL of media for 16 hours (5% CO2, 37°C) prior to beginning the experiment. Cell Stimulation. Following AF or NP cell attachment, the overlying media was replaced with 300 µL of media for stimulation. Four replicates were treated with media alone (negative control), TNFα (25 ng/mL, positive control), IL-17 (100 ng/mL), IFNγ (200 U/mL) or both IL-17 and IFNγ. In a dose-response experiment, a broader range of IL-17 doses (0 - 300 ng/mL) was evaluated with the IFNγ costimulant. Supernatant evaluation. After 72 hours, the supernatant was evaluated for nitric oxide (NOx) release by the Griess reaction and for IL-6 release by ELISA. Cell number was evaluated using an ATP luminescence assay (CellTitreGlo, Promega). Statistical analyses. One-way ANOVA and post-hoc Dunn’s analysis evaluated differences amongst treatment groups for NOx and IL-6 release and cell survival (α = 0.05).

RESULTS.

Stimulation of either AF or NP cells by TNFα expectedly lead to greater production of IL-6 (Figure 1 and Table 1) and NOx (Figure 1 and Table 1) as has been previously published. Stimulation with IL-17 alone provided for elevated production of IL-6 and nitric oxide release in both cell types. Stimulation with IL-17 and IFNγ produced substantial increases in IL-6 production above that of IL-17 alone (~200-fold increases for AF or NP cells) and elevated NOx production for both cell types. Further, a clear dose-response effect was observed for IL-17 in both AF (not shown) and NP (Figure 2) cells. Cell Survival. There was no difference in AF cell survival noted between treatment groups, suggesting that the observed biochemical effects do not reflect variable cell numbers due to proliferation or cell death.

DISCUSSION.

While the impact of macrophage products such as TNFα, IL-1α, and IL-1β have been studied for pathological IVD cells, the impact of lymphocyte-derived immune cytokines is less clear. The results of this study demonstrate that degenerative human IVD cells may respond to IL-17 stimulation with increased production of inflammatory mediators, with a more robust effect observed in the presence of IFNγ. Degenerative and herniated IVD explants are known to contain IFNγ, and in other cell types IL-17 can drive inflammation through the production of multiple cytokines including IL-1β, TNFα, MCP-1, and IL-8, all shown to be relevant to degenerative disc disease. This work demonstrates that the responsiveness of IVD cells to IL-17 and IFNγ is consistent with other cell types, and that these cytokines may contribute to development of IVD pathology. Further investigation will focus on the signaling cascade underlying this synergy.

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REFERENCES.


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